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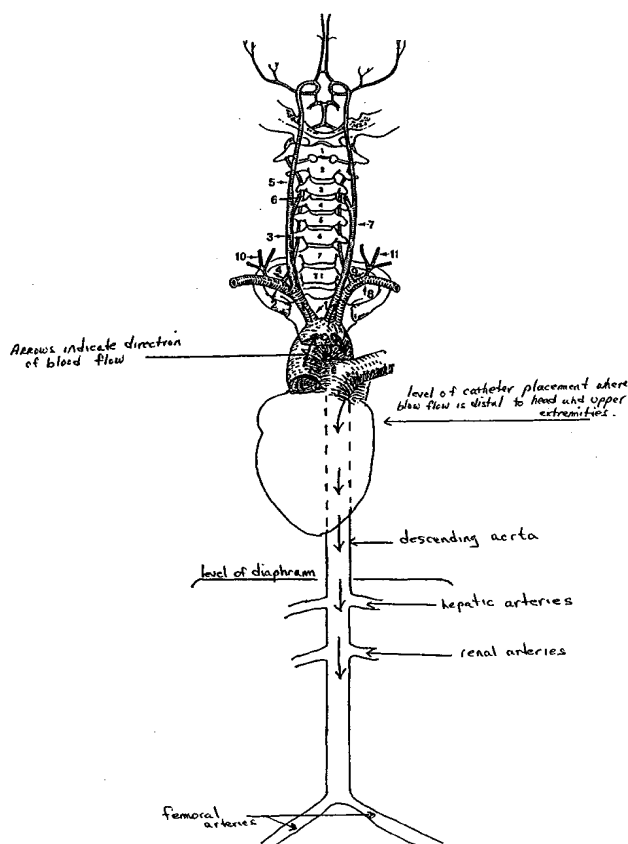
(57) Abstract: A method of administration of a thiol-based chemoprotectant agent including NAC (N-acetylcysteine) and STS (sodium thiosulfate) that markedly affects biodistribution and protects against injury from diagnostic or therapeutic intra-arterial procedures. A method for treating or mitigating the side effects of cytotoxic cancer therapy for tumors located in the head or neck and brain tumors. The thiol-based chemoprotectant agent is administered intra-arterially with rapid and first pass uptake in organs and tissues other than the liver.

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ADMINISTRATION OF A THIOL-BASED CHEMOPROTECTANT COMPOUND

RELATED APPLICATIONS

The present application claims the benefit of the filing dates under 35 U.S.C. § 119(e) to provisional U.S. Patent Application Serial No. 60/199,936 filed on April 26, 2000 and Provisional U.S. Serial No. 60/229,870 filed on August 30, 2000, which are hereby incorporated by reference.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

The present invention was partially supported under NIH grant No. NS33618 and by The Department of Veterans Affairs merit review grant. The United States Government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

The present invention is based, in part, upon the discovery that a method of administration of N-acetylcysteine (NAC) markedly affects its effective biodistribution. The present invention provides a method for treating or mitigating the side effects, including organ damage, of cytotoxic cancer therapy for tumors located in the head or neck. Additionally, NAC or other thiols can be administered concurrently with, before or after, intra-arterial procedures and provides protective affects to prevent or diminish organ damage.

N-acetylcysteine (NAC) is an analog of cysteine. When NAC is administered to a mammal it is deacylated and enters a cellular synthetic pathway for the production of glutathione. Glutathione is involved in the cellular pathways influencing a tumor's resistivity to cytotoxic drugs. The cytotoxic properties of chemotherapeutic drugs can be enhanced by pretreatment with buthionine sulfoximine (BSO) thereby reducing intracellular glutathione. However, reduction of intracellular gluthionine will potentiate systemic toxicities associated with chemotherapeutic drugs. Thus, this procedure is dose-limiting. For protection, the glutathione levels of "normal" cells have to be reestablished if BSO is used to potentiate the cytotoxic properties of cytotoxic cancer therapies (Kamer et al., *Cancer Res.* 47:1593-1597, 1987; Ozols et al., *Biochem. Pharm.* 36:147-153, 1987; McLellan et al., *Carcinogenesis* 17:2099-2106, 1995; and Shattuck et al., *J. Parenteral Enteral Nutrition* 24:228-233, 1998). It may be possible to reduce the bone marrow toxicity of chemotherapeutic drugs by using sulfur-containing chemoprotective agents (thio, thiol, and thioether compounds) to mimic one or many of the activities of glutathione such as conjugation, free radical scavenging, and drug

efflux via the multidrug resistance associated proteins. NAC and other thiol agents such as STS have early detoxifying activity not related to the later increase in glutathione levels. These early detoxifying effects occur because the thiols themselves mimic some actions of glutathione such as free radical scavenging, anti-oxidant activity, chemical conjugation, and activation of efflux pumps.

A potential problem with any chemoprotectant is the possibility of deactivating the anti-tumor effect of the chemotherapy or radiation therapy. The goal of chemoprotection is to reduce unwanted toxicities of chemotherapy or radiotherapy without affecting efficacy.

For brain tumor chemotherapy, one must attempt to increase the delivery of chemotherapy to the brain tumor and block the delivery of the chemoprotective agent. Additionally, one will want to target the chemoprotectant agent to the bone marrow to protect against myelosuppression and to liver, kidney and lung to prevent organ toxicity. Therefore, there is a need in the art to improve pharmacokinetics and biodistribution of chemoprotectant agents so that they will be more effective when delivered in a tissue-specific manner. Preferably, delivery is maximized to the bone marrow, chest and abdomen organs while minimized to the brain.

There are more than 10 specific active transport systems that transport compounds from the blood to the brain. Otherwise, substances, such as chemoprotectants can only nominally penetrate this barrier by passive diffusion. Brain tumors are particularly difficult to treat because the blood-brain barrier is an anatomical structure that limits the egress of constituents in the blood to the brain. Thus, brain tumors often respond poorly to chemotherapeutic drugs. There have been many attempts to try to increase brain bioavailability of various drug compounds to brain tissue. One technique uses osmotic BBB modification by administering mannitol through the internal carotid artery (Neuwelt et al., Cancer Res. 45:2827-2833, 1985). This technique is useful for administering the chemotherapeutic methotrexate to experimental brain tumors that would otherwise be inaccessible to this drug (it poorly crosses the BBB). The osmotic shrinkage caused by intracarotid mannitol administration allowed for temporary BBB disruption and increased tumor delivery of the methotrexate. Thus, a temporary disruption of the barrier functions of the BBB can be induced by a sugar, such as mannitol, and cause higher brain concentrations of a drug compound that would not otherwise have crossed the BBB. This BBB opening technique has also been investigated with other chemotherapeutic drugs (Neuwelt et al., Proc. Natl. Acad. Sci. USA 79:4420-4423, 1982; Fortin D. McCormich Cl, Remsen LG, Nixon R, Neuwelt EA, "Unexpected neurotoxicity of etoposide phosphate when given in combination

with other chemotherapeutic agents after blood-brain barrier modification using propofol for general anesthesia in a rat model," *Neurosurgery* 47:199-207, 2000).

An example of chemoprotection is a drug neutralization technique described in U.S. Patent 5,124,146 wherein excess toxic drug compounds are "mopped up" or bound by a binding or neutralizing agent not able to penetrate the blood brain barrier. This technique requires precise timing as to when the drug neutralizing agent is administered.

There are several thiol-based chemoprotectant agents that contain a thio, thiol, aminothiol or thioester moiety. Several thiol-based chemoprotectant agents have been shown to provide protection against at least some of the systemic toxicities caused by alkylating chemotherapeutics. The thiol based chemoprotective agents include N-acetyl cysteine (NAC), sodium thiosulfate (STS), GSH ethyl ester, D-methionine, and thiol amifostine (Ethyol or WR2721). NAC is currently marketed in the United States under an orphan indication for oral and intravenous (i.v.) administration for overdosing with acetaminophen. NAC has also been shown to be a chemoprotectant when administered in combination with a vanadate compound (U.S. Patent 5,843,481; and Yarbo (ed) *Semin. Oncol.* 10 [Suppl 1]56-61, 1983). Ethyol is also marketed in the United States under the generic name of Amifostine. GSH ethyl ester is an experimental thiol not yet marketed for clinical use, but is representative of the class of thiols that is converted directly to glutathione.

In addition, NAC has been shown to be a mucoregulatory drug used for the treatment of chronic bronchitis (Grassi and Morandini, *Eur. J. Clin. Pharmacol.* 9:393-396, 1976; Multicenter Study Group, *Eur. J. Respir. Dis.* 61: [Suppl.]93-108, 1980; and Borman et al., *Eur. J. Respir. Dis.* 64:405-415, 1983).

In plasma, NAC can be present in its intact, reduced forms as well as in various oxidized forms. It can be oxidized to a disulfide by reacting with other low molecular weight thiols, such as cysteine and glutathione. NAC can be oxidized by reacting the thiol groups of plasma proteins. When administered intravenously, the brain levels of NAC are <5%. Yet, NAC does cross the BBB if given by an intra-arterial route of administration. NAC is rapidly cleared from plasma via the liver and kidney. Moreover, NAC does not show neurotoxic properties.

There are bioanalytical methods for the determination of NAC in plasma, including Cotgreave and Moldeus, *Biopharm. Drug Disp.* 8:365-375, 1987; and Johansson and Westerlund, *J. Chromatogr.* 385:343-356, 1986 that also permit a determination of other forms of NAC. Moreover, cysteine and cystine have been identified as major metabolites of NAC. The excreted urinary product is inorganic sulfate together with small amounts of

taurine and unchanged NAC. According to the label indications for NAC manufactured by (American Regent Laboratories Shirley, NY), vials of NAC are produced as a sterile solution for oral administration diluted with water or soft drinks.

5   Another thiol-containing chemoprotectant is sodium thiosulfate (STS). Its chemical formula is $Na_2S_2O_3$ and it has been used clinically for cyanide poisoning and for nephrotoxicitiy caused by cisplatin. STS is cleared rapidly from circulation primarily by the kidney. The plasma half life after a bolus injection is about 17 minutes. STS can also inactivate platinum agents through covalent binding to platinum agents at a molar excess >40:1 (STS:platinum). With i.v. administration of STS, the brain levels of STS are <5% of

10   blood showing poor brain localization. Neurotoxic side effects, in the form of seizures, may occur when brain levels of STS are enhanced through i.a. administration within 30 min of BBB disruption.

Diagnostic or therapeutic procedures involving intra-arterial catheterization can cause a variety of organ toxicities, complications and side effects from injuries. For example,

15   placement of an arterial catheter can dislodge plaques from artery walls that can lodge elsewhere in the vasculature causing ischemia. Ischemia increases the presence of free radicals and leads to cell death. As another example, nephrotoxicity of radiographic contrast agents can lead to acute renal failure even when measures are taken to reduce toxic effects. As a third example, intra-arterial catheterization is used during angioplasty procedures

20   wherein a balloon catheter is inserted into the arterial circulation and then threaded (with radiographic contrast agents for visualization) to a site of occlusion. In dilating the occluded artery, various forms of tissue damage and inflammatory reactions (*e.g.*, restenosis) can occur including ischemic tissue injury.

Specifically, toxic side effects of intra-arterial catheterization and infusion of

25   radiographic contrast agents prolong hospital stays, add to the cost of medical care, and can be fatal. The incidence of radiographic-contrast-agent-induced acute renal failure, currently estimated to be as high as 50 percent among patients with diabetes mellitus and preexisting renal disease who receive contrast agents, is likely to remain high as the use of invasive intra-arterial procedures to diagnose and treat complex disease continues to grow.

30   Radiographic contrast agents are used in medical imaging. Medical imaging is the production of images of internal organs and tissues by the application of nonsurgical techniques. Contrast agents are chemicals used to enhance the image, and to increase contrast between the target organ and surrounding tissues. Prevention or mitigation of renal failure after the administration of a radiographic contrast agent has been notably difficult.

Calcium-channel antagonists, adenosine antagonists, and dopamine have all been used without convincing evidence of benefit.

Tepel et al. proposed the oral administration of approximately 1200 mg of N-acetylcysteine per day, given orally in divided doses on the day before and on the day of the administration of the radiographic contrast agent. (Tepel et al., *New England J. Med.*, July 20, 2000). Oral administration allegedly prevented the expected decline in renal function in all patients with moderate renal insufficiency, and therefore high risk, who were undergoing computed tomography.

NAC has been used successfully to ameliorate the toxic effects of a variety of experimentally or clinically induced ischemia-reperfusion syndromes of the heart, kidney, lung, and liver. In each of these syndromes, it is thought that the activity of NAC is related to its action as a free-radical scavenger, or as a reactive sulphydryl compound that increases the reducing capacity of the cell. The specific mechanism of NAC to prevent the nephrotoxic effects of contrast agents is not known.

Therefore, there is a need in the art to find better ways to use thiol-based chemoprotectants, such as NAC and STS and to take advantage of their pharmacokinetic properties. There is also a need in the art to find better, higher dose cytotoxic treatment regimens for head and neck as well as brain tumors that avoid dose-limiting due to side effects.

There is a need in the art for a compound that can be used with intra-arterial catheterization procedures to reduce organ toxicity. Diabetic patients with markedly reduced renal function, in whom coronary angiography is often delayed because of the considerable risks to renal function entailed by angiography, may particularly be benefited by targeted delivery of a protectant agent. Additionally, there is a need in the art for a low cost compound which is generally available, easy to administer and has limited side effects. There is a need in the art for a compound and a method of administration of the compound that can be used to reduce or eliminate tissue damage caused by intra-arterial procedures.

Additionally, there is a need in the art to find better ways to use thiol-based radiographic protectants, such as NAC and STS (sodium thiosulfate) and to take advantage of their pharmacokinetic properties. There is also a need in the art to find an agent protective against intra-arterial catheterization-induced reductions in organ function. These and other problems of the prior art are solved by the present method and pharmaceutical composition.

SUMMARY OF THE INVENTION

The present invention provides a method and compound for locally administering a thiol based chemoprotectant to treat or mitigate the side effects of cytotoxic cancer therapy for brain tumors located in the head or neck. Further, the present invention provides a

5   method and compound for locally administering a thiol-based chemoprotectant to an organ or tissue to protect against injury from diagnostic or therapeutic intra-arterial procedures. The method includes administering a thiol-based chemoprotectant agent intra-arterially in conjunction with, before, or after administration of a cytotoxic agent.

In one embodiment, the cytotoxic agent is a cancer chemotherapeutic agent. The

10   cytotoxic agent is dose-limited due to myelosuppressive effects systemically. The cancer chemotherapeutic agent is selected from the group consisting of cis-platinum compounds, taxanes (*e.g.*, paclitaxel), steroid derivatives, anti-metabolites, vinca alkaloids, adriamycin and doxorubicin, etoposide, arsenic derivatives, intercalating agents, alkylating agents (such as melphalan) and combinations thereof. The cytotoxic agent is administered within eight

15   hours (before, during or after) of the thiol-based chemoprotectant agent administration. In yet another embodiment, when the tumor is located in the head or neck or brain, the cytotoxic agent is administered such that the majority of the dose is directed to the head or neck region.

Preferably, the thiol-based chemoprotectant agent is a compound selected from the group consisting of N-acetyl cysteine (NAC), sodium thiosulfate (STS), GSH ethyl ester,

20   D-methionine, Ethyol, and combinations thereof. In one embodiment, the thiol-based chemoprotectant agent is administered in a pyrogen-free sterile solution by a catheterization procedure via a catheter having a tip that is located in the descending aorta. In yet another embodiment, the dose of the thiol-based chemoprotectant agent per procedure is from about 200 mg/m^2 to about 40 g/m^2. Most preferably, the dose of NAC agent per procedure is from

25   about 400 mg/m^2 to about 1200 mg/m^2 and the dose of STS is from about 5 g/m^2 to about 40 g/m^2.

In one embodiment, the intra-arterial catheter is positioned in an artery providing blood flow to a potential site or organ of injury. In yet another embodiment, the intra-arterial administration is at a site in the descending aorta. In yet another embodiment, the injury is

30   caused by injecting a cytotoxic agent including a radiographic contrast agent using an intra-arterial catheter.

In yet another embodiment, the thiol-based chemoprotectant agent is administered in a pyrogen-free, non-oxidized sterile solution having a reducing agent, a buffer to maintain pH

at or near physiologic pH and a metal chelating agent to bind metal ions that can catalyze

oxidation of the thiol-based chemoprotectant agent. Preferably, the reducing agent is selected

from the group consisting of vitamin E, tocopherol, dithiotreitol, mercaptoethanol,

glutathione, and combinations thereof. Preferably, the buffer is one that is relatively

5   non-toxic and can maintain a pH of between 6 and 8 (*e.g.*, phosphate buffer, Tris buffer).

Preferably, the thiol-based chemoprotectant agent is stored in a vial having a blanket of an

inert gas. Most preferably, the inert gas is selected from the group consisting of argon,

helium, nitrogen and mixtures thereof.

The present invention further provides a pharmaceutical composition for treatment to

10   protect against injury from diagnostic or therapeutic intra-arterial procedures, and for head

and neck brain tumors. The compound includes a first agent for intra-arterial administration

and a second agent administered intra-arterially.

Preferably the second agent is a thiol-based chemoprotectant agent. In one

embodiment, the second agent is administered to the descending aorta or further downstream.

15   The first agent is administered to a carotid or vertebral artery. The first agent is a

radiographic contrast agent delivered intra-arterially to position a catheter. In one

embodiment, the first agent is administered within eight hours (before, during or after) of the

second agent. The second agent is administered immediately after intra-arterial

catheterization, prior to radiographic contrast agent, to within eight hours of the radiographic

20   contrast agent.

In one embodiment, the second agent is administered in a pyrogen-free, sterile

solution. In further embodiments, the solution is non-oxidized and has a reducing agent, a

buffer to maintain pH at or near physiologic pH and a metal chelating agent to bind up metal

ions that can catalyze oxidation of the thiol-based chemoprotectant agent. Preferably, the

25   reducing agent is selected from the group consisting of vitamin E, tocopherol, dithiothreitol,

mercaptoethanol, glutathione, and combinations thereof. Preferably, the buffer is one that is

relatively non-toxic and can maintain a pH of between 6 and 8 (*e.g.*, phosphate buffer, Tris

buffer).

In one embodiment, the thiol-based chemoprotectant agent is stored in a vial having a

30   blanket of an inert gas. Most preferably, the inert gas is selected from the group consisting of

argon, helium, nitrogen and mixtures thereof. Preferably, the thiol-based chemoprotectant

agent is a compound selected from the group consisting of N-acetyl cysteine (NAC), sodium

thiosulfate (STS), GSH ethyl ester, D-methionine, Ethyol, and combinations thereof.

Preferably, the dose of the thiol-based chemoprotectant agent per procedure is in the range of

200 mg/m^2 to 2000 mg/m^2. In a further preferred embodiment, the dose of NAC per

procedure is in the range of 400 mg/m^2 to 1200 mg/m^2.

Further, one advantage of NAC is that it is protective against multiple intra-arterial

procedure toxicities, including but not limited to those caused by radiographic contrast

5   agents.

The methods and compounds will best be understood by reference to the following

detailed description of the preferred embodiment, taken in conjunction with the

accompanying drawings. The discussion below is descriptive, illustrative and exemplary and

is not to be taken as limiting the scope defined by any appended claims.

10   BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an anatomical diagram of major arteries and the top level for placing

the catheter for administration of the thiol-based chemoprotectant agent.

Figure 2 (A-C) shows the effect of bone marrow recovery and lower nadirs using the

left carotid aortic infusion inventive method with chemotherapy and the chemoprotectant

15   NAC. Specifically, Figure 2A shows the effect on white blood cells, Figure 2B the effect on

platelets and Figure 2C the effect on granulocytes.

Figure 3A shows dose/response for N-acetylcysteine chemoprotection. Figure 3B

shows dose/response for D-methionine chemoprotection. Cytotoxicity was assessed in cultured

LX-1 SCLC cells, 1×10^4 cells per well in 96 well plates, using the WST colorometric assay.

20   Cells were treated with approximately 90% lethal dose of chemotherapy (melphalan = 20

μg/ml, carboplatin = 200 μg/ml, cisplatin = 20 μg/ml). Chemoprotectant was added at the

indicated concentration of N-acetylcysteine shown in Figure 3A or D-methionine shown in

Figure 3B either alone (n) or immediately following chemotherapy. Data are expressed as the

percentage of live cells compared to untreated control samples (without chemotherapy) and

25   each point represents the mean ± standard deviation of 4 wells.

Figures 4A and 4B show cytoenhancement and chemoprotection. Figure 4A shows the

effect of BSO and N-acetylcysteine on Carboplatin cytotoxicity. Figure 4B shows the effect of

BSO and N-acetylcysteine on etoposide phosphate cytotoxicity. Cytotoxicity was assessed in

cultured LX-1 SCLC cells, 1×10^4 cells per well in 96 well plates, using the WST colorometric

30   assay. BSO cytoenhancement consisted of preincubation at 100 μM BSO for 18 hours. The

chemoprotective agent was added immediately after chemotherapy. The experimental

conditions were dose/responses for chemotherapy (carboplatin or etoposide phosphate) alone (

m), BSO cytoenhancement (Æ), N-acetylcysteine rescue, (1000 μg/ml N-acetylcysteine, n),

or BSO cytoenhancement and N-acetylcysteine rescue (u). Data are expressed as the percentage of live cells compared to untreated control samples without chemotherapy and each point represents the mean ± standard deviation of 4 wells.

Figure 5 shows cytoenhancement and chemoprotection in fibroblasts. Cytotoxicity was assessed in GM294 human fibroblasts, 1×10^4 cells per well in 96 well plates, using the WST colorometric assay. Cells were pretreated with or without BSO, 100 μM for 18 hours prior to addition of chemotherapeutics (melphalan = 10 μg/ml, carboplatin = 100 μg/ml, cisplatin = 7.5 μg/ml, etoposide phosphate 100 μg/ml) either alone (open bar), or with N-acetylcysteine rescue, (1000 μg/ml N-acetylcysteine, striped bar), BSO cytoenhancement (black bar), or BSO cytoenhancement and N-acetylcysteine rescue (cross hatched bar). Data are expressed as the percentage of live cells compared to control samples (without chemotherapy) and each point represents the mean ± s.d. of 4 wells.

Figure 6 shows time dependence for rescue of chemotherapy cytotoxicity. Chemotherapy cytotoxicity was assessed in cultured LX-1 SCLC cells (1×10^4 cells/well in 96 well plates) using the WST colorometric assay. Cells were treated with melphalan at 20 μg/ml, carboplatin 200 μg/ml, cisplatin 10 μg/ml, or etoposide phosphate 200 μg/ml. Cells then received either no protectant (open bars), or sodium thiosulfate, 2000 μg/ml, added immediately (striped bars), 2 hours (black bars) or 4 hours (cross hatched bars) after chemotherapy. Data are expressed as the percentage of live cells compared to control samples (without chemotherapy) and each point represents the mean ± standard deviation of 4 wells.

Figures 7A and 7B show the effect of cytoenhancement and chemoprotection on apoptosis. Figure 7A shows caspase-2 enzymatic activity. Figure 7B shows TUNEL staining for DNA fragmentation. Apoptosis was assessed in cultured LX-1 SCLC cells pretreated for 18 hours with or without 100 μM BSO. The experimental conditions were no addition (open bars), melphalan (10 μg/ml, striped bars) or melphalan (10 μg/ml) plus N-acetylcysteine (1000 μg/ml) (cross hatched bars) for 20 h prior to harvest. Caspase-2 activity is expressed as percentage activity in untreated control samples (mean ± standard deviation, n = 3). TUNEL staining is expressed as the percentage of cells showing positive staining.

Figure 8 shows the results of NAC delivery to rat brain. Radiolabeled NAC in combination with unlabeled low dose NAC (140 mg/kg) or high dose NAC (1200 mg/kg) was administered to rats with the following routes of infusion: i.v. (open bars), intra-arterially into the right carotid artery (striped bars), intra-arterial via the left carotid artery with left internal artery occlusion (aortic infusion) (black bars), and intra-arterial (right carotid) with

BBBD (cross hatched bars). Radiolabel in tissue homogenates is expressed as the mean and standard error of the % administered dose of 14C-NAC per gram of tissue (n = 3 rats per group). Significant differences from i.v. delivery are indicated by *P<.05 and **P<.001.

Figure 9 represents tests showing the effect of NAC route of administration on

5   mortality +/- BSO. A Kaplan-Meier product limit analysis was used to evaluate the mortality due to chemoprotection with NAC. Rats were treated with or without BSO (10 mg/kg i.p. b.i.d. x 3, black bars) prior to treatment with chemotherapy (Carboplatin 200 mg/m2, etoposide phosphate 100 mg/m2, melphalan 10 mg/m2). Chemoprotection consisted of NAC (1200 mg/m2) or NAC (1000 mg/m2) plus STS (8 g/m2) given either i.v. (n = 17 BBSO, n = 8

10   +BSO) or by aortic infusion (n = 19 BBSO, n = 28 +BSO). P=0.0014 by Wilcoxon analysis.

Figures 10A-10D show the results of tests on chemoprotection for chemotherapy-induced myelosuppression. Rats received tri-drug chemotherapy (Carboplatin 200 mg/m2, etoposide phosphate 100 mg/m2, melphalan 10 mg/m2), with (triangles, squares) or without (circles) chemoprotection. Blood counts were determined prior to chemotherapy, at the blood

15   nadir (6 days), and in the recovery phase (9 days after treatment). Chemoprotection was with NAC (1200 mg/kg) administered via aortic infusion 30 min prior to chemotherapy (triangles) or NAC prior to chemotherapy and STS (8 g/m2) immediately after chemotherapy (squares). In Figure 10B and Figure 10D animals received BSO (10 mg/kg i.p. x 3 d) prior to treatment with chemotherapy. Panel A shows granulocyte counts without BSO Figure 10B shows

20   granulocyte counts with BSO (mean +/- SEM, n = 6 per group) Figure 10C shows platlet count without BSO Figure 10D shows platlet count with BSO. Significant difference from the no protectant groups were determined by Wilcoxon/Kruskal-Walliis rank sums tests (* p<0.05, ** p<0.01).

DETAILED DESCRIPTION OF THE INVENTION

25   The present method includes administration of at least two different agents for treating brain or head and neck tumors. The first agent is a cytotoxic agent. In one example, the cytotoxic agent is radiation or a chemotherapeutic agent. The radiation or chemotherapeutic agent generally has a dose-limiting systemic side effect of myelosuppression. If myelosuppression is too severe, it is life threatening as the patient is

30   unable to generate enough white blood cells of multiple lineages to coordinate immune surveillance function for defending against pathogen attack. Therefore, any ability to reduce bone marrow toxicity or myelosuppression will allow for greater and more effective administration of the cytotoxic agent.

Current treatment to reduce bone marrow side effects include recombinant growth factors that are lineage-specific. Such growth factors have included EPO (erythropoietin) for red cells and G-CSF (granulocyte colony stimulating factor) or GM-CSF (granulocyte macrophage colony stimulating factor) for various lineages of infection-fighting white cells.

5 In addition, TPO (thrombopoietin) is in clinical trials for augmenting a platelet response in myelosuppressed patients. However, such growth factors act to stimulate lineage specific precursor cells to divide and mature down lineage-specific paths. Thus, the use of growth factors results in a more rapid recovery from bone marrow toxicity but does not generally reduce the nadir of toxicity. Such growth factors have been able to allow a patient to tolerate

10 a greater number of cytotoxic treatments (where myelosuppression is a limiting toxicity), but generally not higher doses of the cytotoxic agent administered.

The method allows for greater doses of the cytotoxic agent directed to head and neck tumors. Specifically, the cytotoxic agent (if it is a chemical compound or combination of compounds) is administered intra-arterially such that it is directed initially to the head and

15 neck circulation. Thus, the highest concentration of cytotoxic agent is direct to the location of the tumor to be treated. By contrast, iv administration provides for the same concentration of cytotoxic agent in the bone marrow, where side effects happen, as systemic dose.

Thiol-based chemoprotectant agents are nonspecific chemoprotectant agents. They are not specific to "normal" tissue or cells but can protect both normal and tumor tissue.

20 Earlier attempts to utilize the chemoprotectant properties of thiol-based chemoprotectant agents have failed due to the fact that they were administered either orally or systemically, they were rapidly metabolized, and protected both normal and tumor tissue. Systemic administration includes iv.

The method includes utilizing a spatial two-compartment pharmacokinetic model

25 which results in a general tissue first pass effect to prevent significant or chemoprotectant doses of thiol-based chemoprotectant agents from gaining general systemic circulation through the venous circulatory system. The method utilizes only one pass going to tissues below the level of the heart in order to effect a chemoprotectant effect. Therefore, head and neck tumors are treatable through regionalization of doses of the cytotoxic agent to the brain

30 or head and neck where the tumor tissue is located and doses of the chemoprotectant to general tissues below the level of the heart where the majority of bone marrow tissue is located.

The ability of a thiol-based chemoprotectant agent to show a first pass effect through non-liver tissue was surprising. Once any thiol-based chemoprotectant agent that is not

tissue-absorbed gains access to the venous circulation, it will be cleared through a liver first pass or rapidly removed through renal clearance. When administered not according to the method described, NAC is actively transported across the BBB. An example of spatial compartmentalization is the administration of a thiol-based chemoprotectant agent into the

5   descending aorta or lower preventing any significant chemoprotectant concentrations of the thiol-based chemoprotectant agent from ever reaching the brain or head or neck region where the tumor tissue is located. Spatial compartmentalization is facilitated by the rapid tissue uptake of the thiol-based chemoprotectant agent. Spatial compartmentalization is needed in order to achieve any meaningful therapeutic benefit with a thiol-based chemoprotectant

10   agent. Without spatial compartmentalization, the undesirable result of the thiol-based chemoprotectant agent protecting the tumor tissue will occur. In the case of NAC as the thiol-based chemoprotectant agent, passage of the BBB by NAC will restore glutathione levels to the brain tumor, thereby increasing the tumor's resistance to cytotoxic drugs.

The ability to set up a two-compartment pharmacokinetic model was discovered

15   through a series of experiments using *in vitro* and *in vivo* models and administering both chemotherapeutic agents and the thiol-based chemoprotectant agents NAC and STS. However, it should be noted that radiation therapy can be similarly localized or even more easily localized than chemotherapeutic agents. Tissue culture pharmacological studies have shown that treatment with NAC and STS can reduce cell killing by the chemotherapeutics

20   melphalan, carboplatin, and cisplatin. The *in vivo* results show that localized NAC or STS administration resulted in less myelosuppression and faster recovery from chemotherapy. Moreover, synergistic results were observed with the combination of thiol-based chemoprotectant agent NAC and STS providing evidence for a combination of thiol-based chemoprotectant agents to enhance the protective role when the combination of thiol-based

25   chemoprotectant agents is administered according to the inventive process.

As shown in Figure 1, in another embodiment of the method, a thiol-based chemoprotectant agent is administered intra-arterially such that it is directed systemically. This provides the highest concentration of thiol-based chemoprotectant agent to the location of organ damage, for example the kidneys, to protect against reduction of renal function.

30   Oral administration, by contrast, provides for general systemic administration with the concentration of protective agent going elsewhere in the body, mainly the liver, and requiring higher dosages so as to provide a sufficient dose at the kidney (often the site of organ damage for radiographic contrast agents). In addition, the thiol-based chemoprotectant agent is not specific to normal tissue or cells but can protect both normal and tumor tissue.

The method is based upon results obtained utilizing a spatial two-compartment pharmacokinetic model that resulted in a general tissue first pass effect to prevent significant or radiographic-protectant doses of thiol-based radiographic-protectant agents (specifically illustrated are NAC and STS) from gaining general systemic circulation through the venous

5   circulatory system. Thus, there was a surprising need for only one pass going to tissues of the renal system. This result prevented decreased renal function through regionalization of doses of the radiographic agent to the area where radiography is to be performed and doses of the protectant to the renal system.

As shown in Figure 1, a catheter was inserted into the circulatory system generally via

10   the femoral artery. In the first embodiment, a catheter is inserted into the body and administers a dosage of radiographic contrast agent into the body. An effective dosage of the thiol-based protective agent, preferably NAC, is administered at any time from immediately after the intra-arterial catheterization, preferably before contrast agent, to within about eight (8) hours after the administration of the radiographic contrast agent. The catheter for delivery

15   of the radiographic protective agent is preferably inserted into the arterial system downstream of the aorta and directed in the mesenteric artery system. The thiol-based protective agent is introduced into the body at any time within about 5 hours after administration of the radiographic contrast agent to reduce or eliminate renal failure or decrease in renal function associated with administration of a renal contrast agent.

20   In a further embodiment, a catheter is inserted into the circulatory system (*e.g.*, femoral artery) and administers an effective dosage of a thiol-based protective agent into the body. A radiographic contrast agent is administered by the same catheter and is used to position the catheter to the appropriate place for the therapeutic or diagnostic procedure. The thiol-based protective agent is introduced into the body, generally via the same arterial

25   catheter, at any time within about 5 hours before or after administration of the radiographic contrast agent. Preferably, the thiol-based protective is administered via the arterial catheter one or a plurality of times during the procedure.

Pharmaceutical Formulations

Techniques for the formulation and administration of the compounds of the instant

30   application may be found in "*Remington's Pharmaceutical Sciences*" Mack Publishing Co., Easton, PA, latest addition. Suitable routes of administration are intra-arterial.

The compositions and compounds of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, emulsifying, encapsulating, entrapping, or lyophilizing processes. Pharmaceutical compositions for use in

accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations, which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

5   For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers, such as Hank's solution, Ringer's solution, or physiological saline buffer. The compounds may be formulated for parenteral administration by injection, *e.g.,* by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.,* in ampoules or in multi-dose containers, 10   with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulary agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active 15   compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable 20   stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.,* sterile pyrogen-free water, before use. In one embodiment, a reducing agent or an anti-oxidant agent is added to the formulation of the thiol-based protective agent 25   to prevent oxidation of the thiol-based protective agent. The antioxidant may include, but is not limited to, vitamin E, tocopherol, dithiotreitol, mercaptoethanol, glutathione. In one embodiment, an inert or non-oxidizing gas is added to a vial for intra-arterial administration. Examples of such gasses are nitrogen, argon, helium, and combinations/mixtures thereof.

A therapeutically effective dose refers to that amount of the compound that results in 30   a reduction in the development or severity of myelosuppression. In another embodiment, toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical, pharmacological, and toxicological procedures in cell cultures or experimental animals, *e.g.,* for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The

dose ratio between toxic and therapeutic effects is the therapeutic index and it can be

expressed as the ratio between LD_{50} and ED_{50}. Compounds that exhibit high therapeutic

indices are preferred. The data obtained from cell culture assays or animal studies can be

used in formulating a range of dosage for use in humans. The dosage of such compounds lies

5   preferably within a range of circulating concentrations that include the ED_{50} with little or no

toxicity. The dosage may vary within this range depending upon the dosage form employed

and the route of administration utilized. The exact formulation, route of administration and

dosage can be chosen by the individual physician in view of the patient's condition. (See *e.g.*

Fingl et al., 1975, in "*The Pharmacological Basis of Therapeutics*", Ch. 1).

10   The amount of composition administered will, of course, be dependent on the subject

being treated, on the subject's weight, the severity of the affliction, the manner of

administration and the judgment of the prescribing physician.

According to the method, a thiol-based chemoprotectant agent is administered

intra-arterially in order for systemic tissues to be exposed to an initial dose of the thiol-based

15   chemoprotectant agent in high enough concentration to provide a chemoprotective effect and

to bypass the venous circulation and be eliminated by the liver. NAC is actively transported

across the BBB. In one embodiment, to prevent access to the brain, after a transfemoral

carotid/vertebral artery catheter is placed to perfuse the brain, the catheter can then be

subsequently retracted and placed in the descending aorta for NAC infusion, thus performing

20   a single surgical procedure with one catheter. This allows for minimal risk from arterial

catheter procedures only and for high concentrations of NAC to be delivered to peripheral

tissues and organs but not to brain and head.

Synthesis

Each thiol-based chemoprotectant agent, such as NAC or STS, can be synthesized by

25   convention methods and are commercially available as a sterile solution.

Example 1

This example shows the results of an *in vivo* experiment in rats having a catheter

implanted in the descending aorta for NAC administration. The rats were set up for drug

administration by pushing a catheter forward past the junction of the external and internal

30   carotid arteries (toward the aorta), and temporarily sealing off the internal carotid for good

measure so nothing goes to the brain (left carotid aortic infusion method). In patients where

entry is via the femoral artery and the catheter is threaded through the aorta to get to the brain

in the first place, one would just pull the catheter back to the aorta to do the NAC infusion.

Rats were treated with carboplatin (200 mg/m^2), melphalan (10 mg/m^2) and etoposide phosphate (100 mg/m^2). In the NAC animals, NAC was infused with the left carotid aortic infusion method immediately after the chemotherapeutic agents, at concentrations ranging from 400-1200 mg/m^2. White blood cells (wbc) and platelets (plt) were counted at baseline before the experiment and at 6 and 9-11 days after treatment with the chemotherapeutic agents. The animals that did not receive NAC were tested on day 9-10 while the NAC animals were tested on day 10-11. Counts are in 1000s per μl blood. These data in the initial experiment shown in Table 1 provide initial results without a white blood cell effect. The lack of white blood cell effect was not repeated as there were significant effects shown below.

Table 1

chemo alone	wbc base	wbc d6	wbc d9/10	plt base	plt d6	plt d9/10
mean	11.4	0.8	3.2	778	63	164
+/- sd	+/- 4.1	+/- 0.2	+/- 1.6	+/- 233	+/- 59	+/- 91
number	8	4	3	7	4	3

chem +NAC	wbc base	wbc d6	wbc d10/11	plt base	plt d6	plt d10/11
mean	7.9	0.5	4.8	817	101	1232 **
+/- sd	+/- 2.1	+/- 0.2	+/- 0.7	+/- 142	+/- 48	+/- 167
number	5	4	3	5	4	3

The data in table 2 show additional results. In the NAC animals, NAC was infused by the left carotid aortic infusion method 30 min prior to chemo, at a concentration of 1200 mb/m2. White blood cells (wbc) and platelets (plt) were counted at baseline before the experiment and at 6 and 9 days after treatment with the chemotherapeutic agents. The data in Table 1 show that NAC treatment decreased the nadir blood count for both white cells and platelets, and blood counts recovered from chemotherapy faster.

Table 2

chemo alone	wbc base	wbc d6	wbc d9	plt base	plt d6	plt d9
mean	6.4	1.6	5.2	878	187	599
+/- sd	+/- 0.3	+/- 0.4	+/- 0.7	+/- 46	+/- 70	+/- 187
number	6	6	6	6	6	6

chem +NAC	wbc base	wbc d6	wbc d9	plt base	plt d6	plt d9
mean	5.1	3.3	6.2	721	388	1155
+/- sd	+/- 0.4	+/- 0.7	+/- 1.6	+/- 59	+/- 95	+/- 154
number	6	6	6	6	6	6

These data show that with NAC the platelets recovered from chemotherapy faster.

In addition, the experiment with the same chemotherapy agents at the same doses with and without BSO (buthionine sulfoximine) shows improved white blood cell recovery and higher nadirs with the chemoprotectant (NAC alone or with STS at the doses listed above) in Figure 2A. Similar data are shown with platelets in Figure 2B, and with granulocytes in Figure 2C.

Example 2

This example shows the results of NAC delivery by different means of catheter-based administration. In this experiment, radiolabeled NAC was administered to rats by three different routes. Liver and kidney tissue, in addition to the ipsilateral and contralateral hemispheres of the brain, were analyzed for radioactivity. Results are shown as the percent of the injected radioactive dose per gram of tissue. Route indicates intravenous (i.v.), intra-arterial into the right carotid artery (i.a.), or left carotid with internal artery occlusion and aortic infusion (left carotid aortic infusion). The left carotid aortic infusion intra-arterial method uses descending aorta placement of the catheter tip and NAC administration.

route	left hem	right hem	liver	kidney	number
i.v.	0.02	0.03	0.59	0.72	2
i.a.	0.03	0.41	0.57	0.70	3
Left carotid aortic infusion	0.04	0.04	0.29	1.42	2

These data show that i.a. delivery provided much brain delivery in the infused hemisphere. When the NAC was administered i.v., negligible amounts were found in brain (0.03 % of the injected dose, n=2). When radiolabeled NAC was administered intraarterially into the right carotid artery of the rat, high levels of radiolabel were found throughout the right cerebral hemisphere. Delivery was 0.41% of the injected dose (n = 3), comparable to the levels found in liver (0.57 % of the injected dose) or kidney (0.70 % of the injected dose). In contrast, the left carotid aortic infusion method prevented brain delivery. The Left carotid

aortic infusion method also changed bio-distribution in peripheral tissues in that liver delivery was decreased and kidney delivery was increased. The change in tissue delivery with different modes of administration is likely due to NAC being related to the amino acid cysteine that is rapidly bound by tissues via the amino acid transporters. In summary, the method of administration of NAC markedly affected its biodistribution.

Example 3

This example tested whether the inventive method for intra-arterial infusion (via the left carotid artery, with left internal artery occlusion) could reduce brain delivery of NAC and increase systemic delivery. Brain delivery was 0.04 % of the injected dose with the inventive method (n = 2).

In conjunction with other pharmacological and physiological data, these results show that NAC is protective when N-acetylcysteine is administered prior to, (preferably 30 minutes prior to), the cytotoxic agent at a dose which provides a serum concentration of NAC of between 0.5 mM to 15.0 mM, preferably 5 mM to 12.5 mM. Generally, a dose of between 40.0 mg/kg to 1000 mg/Kg of N-acetylcysteine will provide an appropriate serum concentration in humans and other mammals.

Example 4

This example shows the inventive process being used to compare bone marrow toxicity of a chemotherapeutic agent (alone or in combination) to administration of NAC alone (1000-1200 mg/m^2 alone or in combination) and NAC plus STS. The chemotherapy agents were carboplatin (200 mg/m^2) melphalan (10 mg/m^2) and etoposide phosphate (100 mg/m^2). The dose of STS in the rat was 8 g/m^2 that is the equivalent to 20 g/m^2 in humans. The method of administration was left carotid aortic infusion method. These data are expressed according to lineages of bone marrow cell in Table 3a and 3b.

Table 3a

		wbc	wbc	wbc	Platelet	Plt	plt
chemo	mean	9.2	0.7	3.2	759.0	63.3	164.0
	sd	3.6	0.2	1.6	260.5	59.0	91.0
	n	7	4	3	7	4	3
		wbc	wbc	Wbc	Platelet	Plt	plt
chemo +	mean	7.1	1.4	5.2	772.3	132.0	1559.0
	sd	2.7	1.1	1.7	171.5	36.8	567.1
	n	4	2	2	4	2	2
		wbc	wbc	wbc	Platelet	Plt	plt
chemo +	mean	9.5	2.8	8.6	936.5	554.0	1950.0
NAC +	sd	6.0	0.4	3.6	186.0	147.1	134.4

n	2	2	2	2	2	2

Table 3b

chemo+ BSO	wbc base	wbc d6	wbc d9	plt base	plt d6	plt d9
mean	6.8	0.8	5.4	930	92	387
+/- sd	+?- 0.8	+/- 0.2	+/- 1.1	+/- 63	+/- 28	+/- 139
number	11	11	6	11	11	6

BSO+chem +NAC	wbc base	wbc d6	wbc d9	plt base	plt d6	plt d9
mean	8.4	2.2	7.6	818	341	1560
+/- sd	+/- 1.5	+/- 0.3	+/- 1.2	+/- 99	+/- 79	+/- 194
number	9	9	6	9	9	6

These data show the synergistic effect of a combination of STS and NAC as the combined thiol-based chemoprotectant agents administered according to the inventive process.

Example 5

This example provides the results of an *in vitro* experiment using a combination of a taxane (paclitaxel) with a paclitaxel-cytotoxic enhancing agent BSO and a glutathione-reviving agent NAC in cultured tumor cells. Paclitaxel is cytostatic in cultured cells at concentrations from 1 to 10 micromolar, that is, the tumor cells do not grow but they are not killed either. At 20 uM, paclitaxel begins to be cytotoxic and at 30 uM it is completely toxic in cultured tumor cells. When the tumor cells were pretreated cells with BSO, paclitaxel addition was completely cytotoxic *in vitro* at doses as low as 5 uM.

NAC did not change the dose response for paclitaxel alone. However, NAC completely reversed the enhanced toxicity from the BSO treatment, returning paclitaxel concentration effects to the non-BSO level. This experiment was repeated twice with the data provided in Table 4.

5

Table 4

Treatment	paclitaxel dose	live cells (WST fluorescence)
paclitaxel alone	5	1.274 +/- .071
	20	0.771 +/- .056
paclitaxel + NAC	5	1.369 +/- .061
	20	0.823 +/- .094
BSO + paclitaxel	5	0.056 +/- .004 **
	20	0.045 +/- .004 **
BSO + paclitaxel + NAC	5	1.419 +/- .095 *
	20	0.732 +/- .100 *

15   * significantly different from BSO and paclitaxel

** significantly different from paclitaxel alone

Example 6

Chemoprotection against cytotoxicity

20   The dose/response for rescue from chemotherapy cytotoxicity was evaluated for four different small molecular weight sulfur containing chemoprotectants. Each chemotherapeutic agent was used at a concentration affording approximately 90% lethality in the absence of BSO (20 µg/ml melphalan, 200 µg/ml carboplatin, 15 µg/ml cisplatin). Over all, N-acetylcysteine was the most effective of the thiol agents tested, on a µg/ml basis. The

25   concentration dependence for protection with N-acetylcysteine in comparison to D-methionine is shown in Figures 3A and 3B, and Table 5 shows the EC50 for protection afforded by each protective agent. As shown in Figure 3A AND Table 5, the cytotoxicity of each alkylator was reduced by 75-90% by concurrent administration of N-acetylcysteine, but N-acetylcysteine was more active against melphalan (EC50 = 74 ± 18 µg/ml) than the

30   platinum agents. In contrast, as shown in Figure 3B and Table 5, D-methionine did not protect against melphalan toxicity at the doses tested (50 to 1000 µg/ml), although it was highly protective against cisplatin toxicity, with a half-maximal concentration of 140 ± 41 µg/ml. The maximum magnitude of protection was variable between experiments, ranging

from about 70% to 100% protection, and protection was consistently less for carboplatin than

for cisplatin or melphalan. All agents tested required a significantly higher dose to protect

against carboplatin than against cisplatin or melphalan. On a μg/ml basis, glutathione ethyl

ester was the least effective protective agent.

5   Chemotherapy agents were fixed at the 90% lethal dose for each agent. Each EC50

measurement comprised 6 concentrations with 4 wells per concentration, and each dose

response was performed twice for each protectant. EC50 concentrations, in μg/ml are

reported as the average ± pooled standard deviation for two independent experiments. For

the combination of D-methionine with melphalan, protection was not detected. For each

10   chemoprotectant, t test comparisons were done for melphalan versus cisplatin, cisplatin

versus carboplatin, and carboplatin versus melphalan, and significant differences are

indicated (** = P<0.01; *** = P<0.001).

TABLE 5

15   Protection against chemotherapy cytotoxity

chemotherapy	Chemoprotective agent EC50 (μg/ml)			
	N-acetylcysteine	Sodium thiosulfate	D-methionine	Glutathione ethyl ester
Melphalan	74 ± 18 ***	110 ± 78	None ***	303 ± 124 ***
Cisplatin	151 ± 15 **	86 ± 76 **	140 ± 41 ***	530 ± 30 ***
Carboplatin	200 ± 15 ***	442 ± 203 **	379 ± 123 ***	995 ± 48 ***

Example 7

20   <u>Cytoenhancement and chemoprotection in combination</u>

         The effects of BSO cytoenhancement and thiol chemoprotection on the dose/response

relationships for cytotoxicity of the alkylating chemotherapeutics were evaluated in the B.5

LX-1 cells. BSO cytoenhancement consisted of preincubation with 100 μM BSO for about

18-24 hours prior to addition of chemotherapy, and rescue consisted of 1000-2000 μg/ml of

25   thiol chemoprotectant added immediately after chemotherapy. As shown in Figure 4A and

Table 6, BSO consistently decreased the EC50 for cytotoxicity and increased the maximum

degree of toxicity. The specific case of carboplatin and N-acetylcysteine is shown in Figure

4A. Glutathione depletion with BSO increased carboplatin cytotoxicity, reducing the EC50

by 48% (P<0.01). As detailed in Table 6, similar BSO cytoenhancement was found with

30   melphalan (53% reduction of EC50, P<0.001), while the EC50 for cisplatin was reduced only

29% (P<0.05). Chemoprotection with N-acetylcysteine blocked carboplatin toxicity as well as BSO-enhanced cytotoxicity. Similar chemoprotection was found with additional thiol agents, sodium thiosulfate and glutathione-ethyl ester, but D-methionine was only effective against the platinum agents.

Cytoenhancement and chemoprotection against non-alkylating chemotherapeutic agents was also evaluated. As shown in Figure 4B, Glutathione depletion with BSO did not increase the cytotoxicity of etoposide phosphate, nor did N-acetylcysteine decrease the cytotoxicity of etoposide phosphate. Similarly, no enhancement or protection was found with methotrexate or doxorubicin in the B.5 LX-1 cells, although carcinoma cells of gastric origin showed some enhancement with BSO (data not shown). Interestingly, although the growth inhibitory dose of taxol (approximately 10 nM) was not altered by BSO, glutathione depletion did shift the cytotoxic dose of taxol from 15 µM to 2 µM, and this enhanced cytotoxicity was completely reversed with N-acetylcysteine (not shown).

Whether the cytoenhancement and chemoprotection seen in the B.5 LX-1 SCLC cells was a generalized phenomenon was evaluated by testing similar experimental conditions in the GM294 human fibroblast cell strain. Cells were treated with or without chemotherapy at the approximately half maximal dose found in the B.5 LX-1 cells, with or without pretreatment with BSO. As shown in Figures 3A and 3B, although melphalan, cisplatin and carboplatin were all somewhat more cytotoxic in the fibroblasts as compared to the tumor cells, nevertheless BSO enhanced the toxicity of all three alkylators. In fibroblasts, N-acetylcysteine was partially to completely chemoprotective against the cytotoxicity induced by melphalan, cisplatin and carboplatin, independent of BSO treatment. As shown in Figure 5, neither BSO cytoenhancement nor N-acetylcysteine chemoprotection affected the cytotoxicity of etoposide phosphate in fibroblasts.

Half-maximal cytotoxic concentrations (EC50) are expressed as µg/ml. Each EC50 measurement comprised 6 concentrations with 4 wells per concentration, and each dose response was performed in triplicate for each chemotherapeutic agent. Data are reported as the mean ± pooled standard deviation for three independent experiments. P values are shown for the reduction in EC50 by BSO treatment (* = P < 0.05, ** = P < 0.01, *** = P < 0.001).

TABLE 6

Effect of BSO on the cytotoxicity of alkylating chemotherapeutics

Chemotherapy EC50 (µg/ml)

	Melphalan	Cisplatin	Carboplatin
Without BSO	13.8 ± 1.8	8.9 ± 2.4	103 ± 21
+ BSO	6.4 ± 0.6 ***	6.4 ± 0.9 *	55 ± 15 **

Example 8

Time dependence for chemoprotectant rescue from chemotherapy cytotoxicity

How long the addition of chemoprotectant could be delayed after treatment with chemotherapy and remain effective was evaluated. Cells were treated with doses of chemotherapy providing approximately 90% lethality, for melphalan (20 µg/ml), carboplatin (200 µg/ml), or cisplatin (15 µg/ml). The thiol chemoprotectants were added either concurrently with chemotherapy or up to 8 hours after chemotherapy. For melphalan, chemoprotection was reduced if administration of STS was delayed for 2 hours, whereas sodium thiosulfate was still protective for the platinum chemotherapeutics if delayed up to 4 hours after treatment as shown in Figure 6. Similarly, delayed administration of N-acetylcysteine and glutathione ethyl ester reduced their protective activity against melphalan cytotoxicity, whereas both agents maintained protective activity against platinum cytotoxicity. Separately, all three agents were completely protective if added within 1 hour of melphalan, rather than 2 hours as shown in Figure 6. Chemoprotection was not effective against etoposide phosphate cytotoxicity at any time point.

The time dependence of D-methionine rescue of cisplatin cytotoxicity was also evaluated. Unlike chemoprotection with thiosulfate, N-acetylcysteine, or glutathione ethyl ester, the protection afforded by D-methionine was significantly reduced by delayed administration. If delayed for 2 hours after cisplatin, D-methionine protection was reduced by 41.2 ± 10.2 % compared to the maximal protection seen with simultaneous addition, while delaying D-methionine to 4 hours reduced protection by 66.1 ± 4.5 % compared with simultaneous addition. Pretreatment with D-methionine for 30 min prior to addition of cisplatin did not increase the amount of protection compared to simultaneous addition.

Example 9

Effects of cytoenhancement and chemoprotection on apoptosis

Apoptosis was evaluated by measuring Caspase-2 enzymatic activity and by in situ TUNEL staining. Treatment of B.5 LX-1 cells with melphalan resulted in an increase in Caspase-2 activity that was amplified by BSO pretreatment at low melphalan concentrations as shown in Figure 7A. The increase in caspase activity was variable between experiments and ranged from 50-100% at 7-8 h to 250-600% at 20-24 h after treatment with melphalan.

TUNEL staining also demonstrated melphalan-induced apoptosis. In the experiment shown in Figure 7B, TUNEL staining after melphalan treatment was positive in 29 of 3643 cells, compared to 7 of 4395 cells in the untreated control, and BSO treatment prior to melphalan increased the positive staining to 800 of 1699 cells. In both the caspase-2 assay as shown in Figure 7A and the TUNEL staining assay as shown in Figure 7B, the effect of melphalan on apoptosis was reduced by the chemoprotectant N-acetylcysteine. In both assays, activity was maximal with low doses of melphalan, or with a 1 hour pulse treatment with the doses used in the cytotoxicity assays. Continuous treatment with the cytotoxic dose of melphalan actually reduced caspase-2 activity and TUNEL staining.

Cisplatin and carboplatin were less effective than melphalan at inducing caspase activity. Over a range of doses (100, 150, or 200 µg/ml carboplatin, and 5, 10, or 15 µg/ml cisplatin) and times (8, 12, 16, 20, 24 h), each platinum agent increased Caspase-2 activity by 50-100%. No significant amplification of caspase activity was induced by BSO treatment. Additionally, no reduction in caspase enzymatic activity could be detected after addition of N-acetylcysteine, and in some experiments treatment with N-acetylcysteine actually increased cisplatin- or carboplatin-induced caspase activity. Samples of the cells used in the caspase and TUNEL assays were also evaluated for membrane permeability by trypan blue exclusion. In experiments producing negative results with the caspase-2 or TUNEL assays, trypan blue exclusion showed high numbers of non-viable cells after treatment with carboplatin or cisplatin and this was increased by BSO treatment.

Example 10

Biodistribution of radiolabeled NAC

The method of administration of NAC and its biodistribution was tested. Intra-arterial infusion retrograde via the left external carotid artery, with transient left internal artery occlusion, was evaluated as a mechanism to essentially perfuse via the descending aorta with limited delivery to the brain. When NAC was administered i.v., negligible amounts were found in brain as determined by the percent administered dose of 14C-NAC per gram of tissue as shown in Figure 8. Intra-arterial delivery in the right internal carotid artery resulted in high levels of radiolabel in the right cerebral hemisphere, and this was not increased by BBBD. However, aortic infusion minimized brain delivery of NAC as shown in Figure 8. At the low dose of NAC (140 mg/kg), aortic infusion decreased liver delivery and increased kidney delivery, where as there was no change in liver and kidney delivery at high dose (1200 mg/kg) NAC (data not shown). The serum concentration of NAC 10 min after administration of 1200 mg/kg was 2.4 0.6 mg/ml (n = 6) as determined by radiolabel

remaining in the blood.

Example 11

<u>Toxicity of NAC</u>

A dose escalation of NAC was performed in the rat. Initial doses of 140-800 mg/kg
NAC administered i.v. immediately after chemotherapy (n = 4), were well tolerated but
provided no detectable bone marrow protection. Doses of NAC above 1200 mg/kg (n = 3)
were toxic whereas there was no toxicity at 1200 mg/kg. Therefore, 1200 mg/kg of NAC was
used for the bone marrow protection studies, except when administered in conjunction with
STS where a dose of 1000 mg/kg was used due to volume considerations.

The toxicity of NAC was determined when infused for bone marrow protection. As
shown in Figure 9. the mortality due to NAC was significantly dependent on the route of
administration, with i.v. administration significantly more toxic than aortic infusion (P =
0.0014). Pretreatment with BSO markedly enhanced the toxicity of NAC. Groups given i.v.
NAC after BSO treatment were halted early due to excessive mortality, with a stopping rule
of 75% mortality within n = 4 animals. In selected animals (n = 21) from all groups, blood
pressure monitoring indicated most animals that expired experienced persistent acute
hypotension, suggesting cardiac toxicity. Of particular note, however, there was no mortality
nor any evidence of toxicity in 12 animals, without BSO, giving 1200 mg/kg NAC by aortic
infusion 30 minutes prior to chemotherapy.

Example 12

<u>Effect of NAC on chemotherapy-induced bone marrow toxicity</u>

Chemoprotection against chemotherapy-induced bone marrow toxicity was
determined in BSO treated and untreated animals given i.a. carboplatin, etoposide phosphate,
and melphalan. NAC with or without STS was administered either about 30 minutes prior to
chemotherapy or immediately after chemotherapy, and was administered either i.v. or by
aortic infusion. Chemoprotection was found with NAC as shown by increased blood counts
(white blood cells, granulocytes, platelets) at the nadir, compared to no chemoprotectant as
shown in Table 7 and Table 8. Chemoprotection was effective whether or not animals were
pretreated with BSO as shown in Figure 10, and the magnitude of the chemotherapy-induced
bone marrow toxicity nadir was minimized and recovery to normal platelet levels was
improved.

TABLE 7

Protection against chemotherapy-induced myelosuppression

| Treatment | Rats | White Cell | Granulocyte | Platelet Nadir |

		Nadir	Nadir	
No protectant	N = 6	24.5 ± 5.8	20.7 ± 11.0	22.7 ± 8.7
NAC i.v. post chemo	N = 6	46.0 ± 6.5*	57.9 ± 8.8*	36.8 ± 10.4
NAC i.v. 30 min prior	N = 8	51.4 ± 9.4	93.5 ±28.1	53.3 ± 12.7
NAC aortic infusion post chemo	N = 6	25.9 ± 4.9	36.6 ± 17.0	26.8 ± 9.3
NAC aortic infusion 30 min prior	N = 6	69.7 ± 19.3	206.2 ± 125.1*	59.3 ± 17.9
NAC aortic infusion 30 min prior + STS post chemo	N = 6	53.8 ± 12.8*	83.4 ± 21.3*	47.0 ± 11.5

The mean and standard error are shown for nadir blood counts as a percent of baseline.

* indicates $P<0.05$ compared to no protectant, by Wilcoxon/Kruskal-Wallis rank sums tests.

TABLE 8

Protection against BSO-enhanced chemotherapy-induced myelosuppression

Treatment	Rats	White Cell Nadir	Granulocyte Nadir	Platelet Nadir
No protectant	N = 11	12.9 ± 3.5	3.5 ± 1.3	9.1 ± 2.3
NAC aortic infusion post chemo	N = 7	13.9 ± 4.2	19.8 ± 14.7	11.7 ± 3.5
NAC aortic infusion 30 min prior	N = 9	20.1 ± 2.7	115.4 ± 68.8*	23.1 ± 6.2
NAC aortic infusion 30 min prior + STS post chemo	N = 7	30.5 ± 6.5*	121.0 ± 40.2*	39.0 ± 7.4*

The mean and standard error are shown for nadir blood counts as a percent of baseline.

* indicates $P<0.05$ compared to no protectant, by Wilcoxon/Kruskal-Wallis rank sums tests.

In animals that did not undergo BSO treatment, pretreatment with NAC (1200 mg/kg) by aortic infusion, with or without follow-up with STS, was the best treatment strategy as shown in Figures 10A and 10C. As shown in Figure 10A, the chemotherapy-induced decrease in granulocyte counts was completely blocked ($p<0.05$) and platelet toxicity was reduced as shown in Figure 10C by aortic infusion of NAC 30 minutes prior to chemotherapy.

In animals pretreated with BSO, good protection for granulocytes was provided by aortic infusion of NAC about 30 min prior to chemotherapy, but the best protection and the least mortality was provided by NAC aortic infusion before and STS immediately after chemotherapy as shown in Figures 10B and 10D. As shown in Figure 10B, the combination chemoprotection (NAC and STS) regimen significantly blocked the toxicity for granulocytes ($p<0.01$) and, as shown in Figure 10D, platelets ($p<0.01$) compared to animals that received

no chemoprotection. Chemoprotection also significantly enhanced the platelet recovery from chemotherapy. STS alone did not give consistent bone marrow protection (data not shown).

As noted, the discussion above is descriptive, illustrative and exemplary and is not to be taken as limiting the scope defined by any appended claims.

We claim:

1.   A method for treating or mitigating the side effects of cytotoxic cancer therapy for tumors located in the brain, head or neck, comprising administering a thiol-based chemoprotectant agent intra-arterially, wherein the thiol-based chemoprotectant agent intra-arterial administration is at a site in the descending aorta or further downstream from the descending aorta, and a cytotoxic agent.

2.   The method for treating or mitigating the side effects of cytotoxic cancer therapy for tumors located in the brain, head or neck of claim 1 wherein the cytotoxic agent is a cancer chemotherapeutic agent.

3.   The method for treating or mitigating the side effects of cytotoxic cancer therapy for tumors located in the brain, head or neck of claim 2 wherein the cancer chemotherapeutic agent is selected from the group consisting of cis-platinum compounds, taxanes, steroid derivatives, anti-metabolites, vinca alkaloids, adriamycin and doxarubicin, etoposide, arsenic derivatives, intercalating agents, alkylating agents, and combinations thereof.

4.   The method for treating or mitigating the side effects of cytotoxic cancer therapy for tumors located in the brain, head or neck of claim 1 wherein the cytotoxic agent (radiation or chemotherapeutic agent) is administered within eight hours of the thiol-based chemoprotectant agent administration.

5.   The method for treating or mitigating the side effects of cytotoxic cancer therapy for tumors located in the brain, head or neck of claim 1 wherein the cytotoxic agent is administered such that the majority of the dose is directed to the brain, head or neck region where the tumor is located.

6.   The method for treating or mitigating the side effects of cytotoxic cancer therapy for tumors located in the brain, head or neck of claim 1 wherein the thiol-based chemoprotectant agent is administered in a pyrogen-free sterile solution by a catheterization procedure via a catheter having a tip that is located in the descending aorta.

7.   The method for treating or mitigating the side effects of cytotoxic cancer therapy for tumors located in the head or neck of claim 1 wherein the dose of the thiol-based chemoprotectant agent per procedure is from about 200 mg/m^2 to about 20 g/m^2.

8.   The method for treating or mitigating the side effects of cytotoxic cancer therapy for tumors located in the head or neck of claim 1 wherein the thiol-based chemoprotectant agent is selected from the group consisting of N-acetyl cysteine (NAC), sodium thiosulfate (STS), GSH ethyl ester, D-methionine, Ethyol, and combinations thereof.

9.   The method for treating or mitigating the side effects of cytotoxic cancer therapy for tumors located in the brain, head or neck of claim 8 wherein the dose of NAC agent per procedure is from about 400 mg/m^2 to about 1200 mg/m^2 and the dose of STS agent is from about 5 g/m^2 to about 40 g/m^2.

10.   A pharmaceutical composition for treatment of brain, head or neck tumors for administration via arterial catheter comprising: a first agent administered to a carotid artery; and a second agent administered to the descending aorta or further downstream, wherein the first agent is a cytotoxic compound that is used for cancer chemotherapy and causes myelosuppressive side effects systemically, and the second agent is a thiol-based chemoprotectant agent.

11.   The pharmaceutical composition for treatment of brain, head or neck tumors for administration via arterial catheter of claim 10 wherein the first agent is selected from the group consisting of cis-platinum compounds, taxanes, steroid derivatives, anti-metabolites, vinca alkaloids, adriamycin and doxarubicin, etoposide, arsenic derivatives, intercalating agents, alkylating agents, and combinations thereof.

12.   The pharmaceutical composition for treatment of brain, head or neck tumors for administration via arterial catheter of claim 10 wherein the first agent is administered within eight hours (before, during or after) of the second agent and the second agent is administered in a pyrogen-free sterile solution.

13.   The pharmaceutical composition for treatment of head or neck tumors for administration via arterial catheter of claim 10 wherein the dose of the thiol-based chemoprotectant agent per procedure is from about 400 mg/m^2 to about 1200 mg/m^2.

14.   The pharmaceutical composition for treatment of head or neck tumors for administration via arterial catheter of claim 10 wherein the thiol-based chemoprotectant agent is selected from the group consisting of N-acetyl cysteine (NAC), sodium thiosulfate (STS), GSH ethyl ester, D-methionine, Ethyol, and combinations thereof.

15.   The pharmaceutical composition for treatment of head or neck tumors for administration via arterial catheter of claim 14 wherein the dose of NAC agent per procedure is from about 400 mg/m^2 to about 1200 mg/m^2.

16.   A method for locally administering a thiol-based chemoprotective agent to an organ or tissue area to protect against injury from diagnostic or therapeutic intra-arterial procedures comprising: positioning an intra-arterial catheter in an artery providing blood flow to a potential site or organ of injury; and administering, via the positioned arterial catheter, a thiol-based chemoprotectant agent.

17.   The method for locally administering a thiol-based chemoprotective agent to an organ or tissue area to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 16 wherein the injury is caused by injecting radiographic contrast agent to position an intra-arterial catheter.

18.   The method for locally administering a thiol-based chemoprotective agent to an organ or tissue area to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 16 wherein the thiol-based chemoprotectant agent is a compound selected from the group consisting of N-acetyl cysteine (NAC), sodium thiosulfate (STS), GSH ethyl ester, D-methionine, ethyol, and combinations thereof.

19.   The method for locally administering a thiol-based chemoprotective agent to an organ or tissue area to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 16 wherein the thiol-based contrast-protectant agent is administered in a pyrogen-free, non-oxidized sterile solution by a catheterization procedure via a catheter having a tip that is located in intra-arterially in a position of the circulatory system.

20.   The method for locally administering a thiol-based chemoprotective agent to an organ or tissue area to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 16 wherein the dose of the thiol-based chemoprotectant agent per procedure is in the range of 200 mg/m2 to 40 g/m2.

21.   The method for locally administering a thiol-based chemoprotective agent to an organ or tissue area to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 18 wherein the dose of NAC agent per procedure is in the range of 400 mg/m^2 to 1200 mg/m^2 and the dose of STS is in the range of 5 g/m^2 to 40 g/m^2.

22.   The method for locally administering a thiol-based chemoprotective agent to an organ or tissue area to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 16 wherein the thiol-based chemoprotectant agent is administered in a pyrogen-free, non-oxidized sterile solution having a reducing agent, a buffer to maintain pH at or near physiologic pH and a metal chelating agent to bind up metal ions that can catalyze oxidation of the thiol-based chemoprotectant agent.

23.   The method for locally administering a thiol-based chemoprotective agent to an organ or tissue area to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 22 wherein the reducing agent is selected from the group consisting of vitamin E, tocopherol, dithiotreitol, mercaptoethanol, glutathione, and combinations thereof.

24.   The method for locally administering a thiol-based chemoprotective agent to an organ or tissue area to protect against injury from diagnostic or therapeutic intra-arterial

procedures of claim 22 wherein the buffer is one that is relatively non-toxic and can maintain a pH of between 6 and 8.

25.   The method for locally administering a thiol-based chemoprotective agent to an organ or tissue area to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 16 wherein the thiol-based chemoprotectant agent is stored in a vial having a blanket of an inert gas.

26.   The method for locally administering a thiol-based chemoprotective agent to an organ or tissue area to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 25 wherein the inert gas is selected from the group consisting of argon, helium, nitrogen and mixtures thereof.

27.   A pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures, comprising a first agent or procedure for intra-arterial administration and a second agent administered intra-arterially, wherein the second agent further comprises a reducing agent or an anti-oxidant.
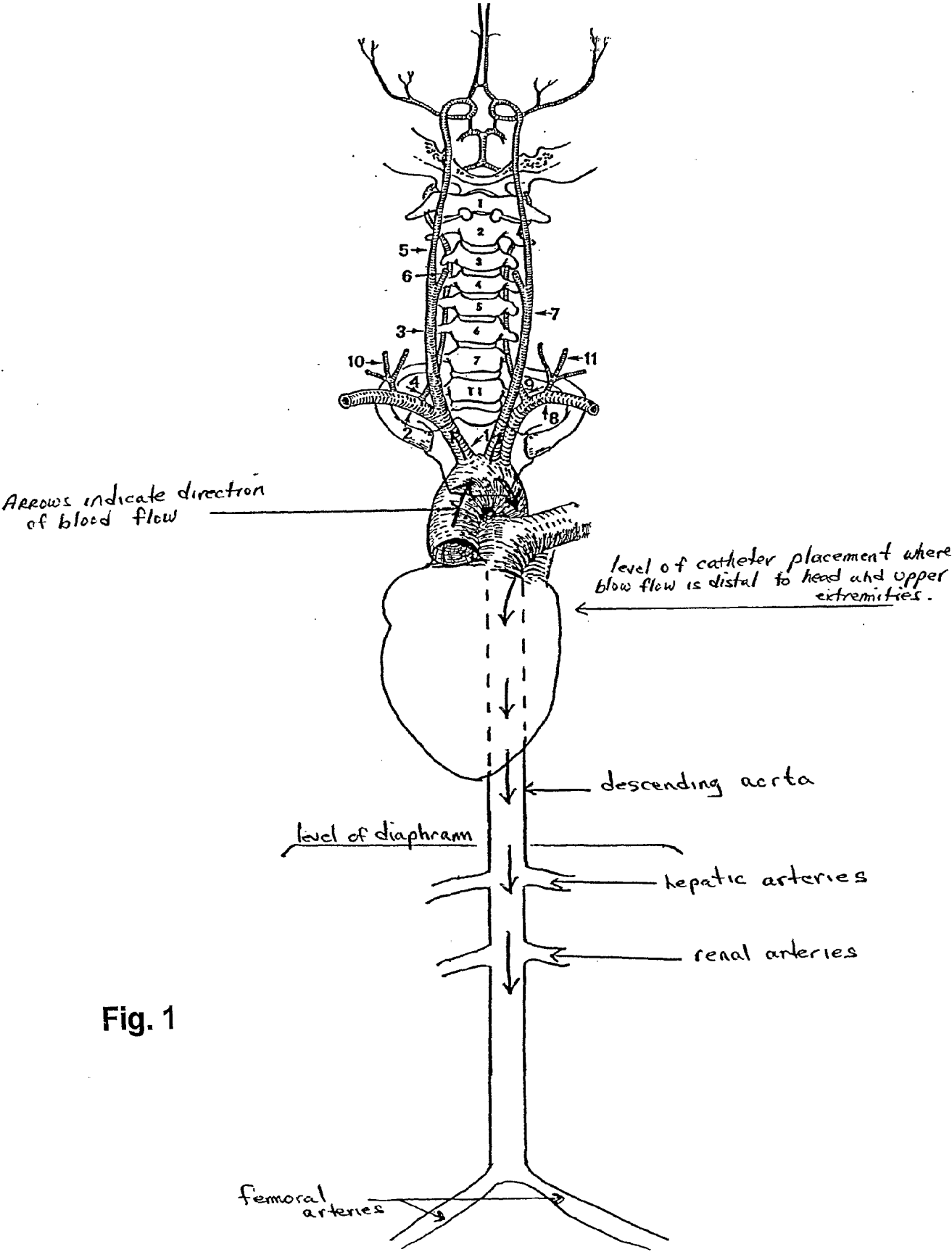
28.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 27 wherein the first agent is a radiographic contrast agent.

29.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 27 wherein the first agent is administered within eight hours of the second agent.

30.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 27 wherein the second agent is administered in a pyrogen-free sterile solution.

31.   The pharmaceutical composition of claim 27 wherein the second agent is a thiol-based chemoprotectant agent.

32.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 31 wherein the thiol-based protectant agent is a compound selected from the group consisting of N-acetyl cysteine (NAC), sodium thiosulfate (STS), GSH ethyl ester, D-methionine, Ethyol, and combinations thereof.

33.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 31 wherein the dose of the thiol-based chemoprotectant agent per procedure is in the range of 200 mg/m^2 to 2000 mg/m^2.

34.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 32 wherein the dose of NAC per procedure is in the range of 400 mg/m^2 to 1200 mg/m^2.

35.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 31 wherein the thiol-based chemoprotectant agent is administered in a pyrogen-free, non-oxidized sterile solution having a reducing agent.

36.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 31 wherein the thiol-based chemoprotectant agent is administered in a buffer to maintain pH at or near physiologic pH.

37.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 31 wherein the thiol-based chemoprotectant agent is administered with a metal chelating agent to bind up metal ions that can catalyze oxidation of the thiol-based chemoprotectant agent.

38.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 35 wherein the reducing agent is selected from the group consisting of vitamin E, tocopherol, dithiotreitol, mercaptoethanol, glutathione, and combinations thereof.

39.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 36 wherein the buffer is one that is relatively non-toxic and can maintain a pH of between 6 and 8.

40.   The pharmaceutical composition for treatment to protect against injury from diagnostic or therapeutic intra-arterial procedures of claim 31 wherein the thiol-based chemoprotectant agent is stored in a vial having a blanket of an inert gas.

41.   The pharmaceutical composition of claim 40 wherein the inert gas is selected from the group, consisting of nitrogen, argon, helium and combinations thereof.

42.   The method for locally administering thiol-based chemoprotectant agent to an organ or tissue wherein the organ or tissue is selected from the group consisting of lungs, liver, kidneys.
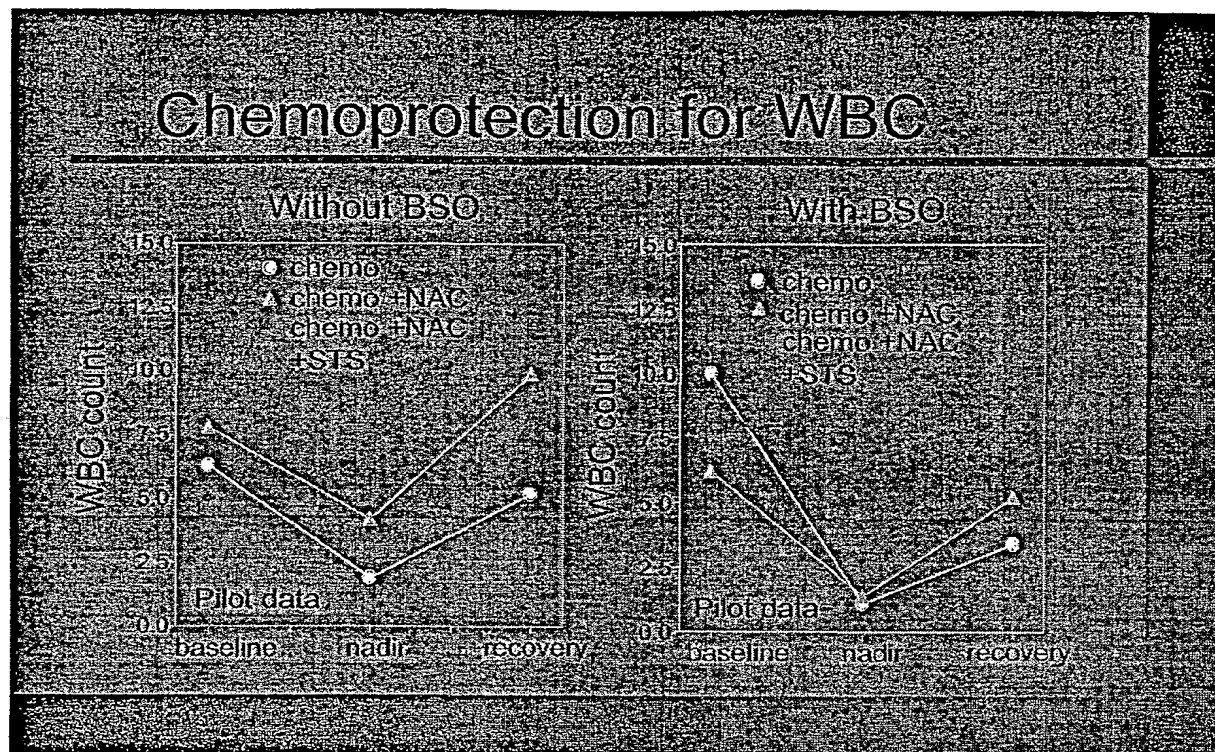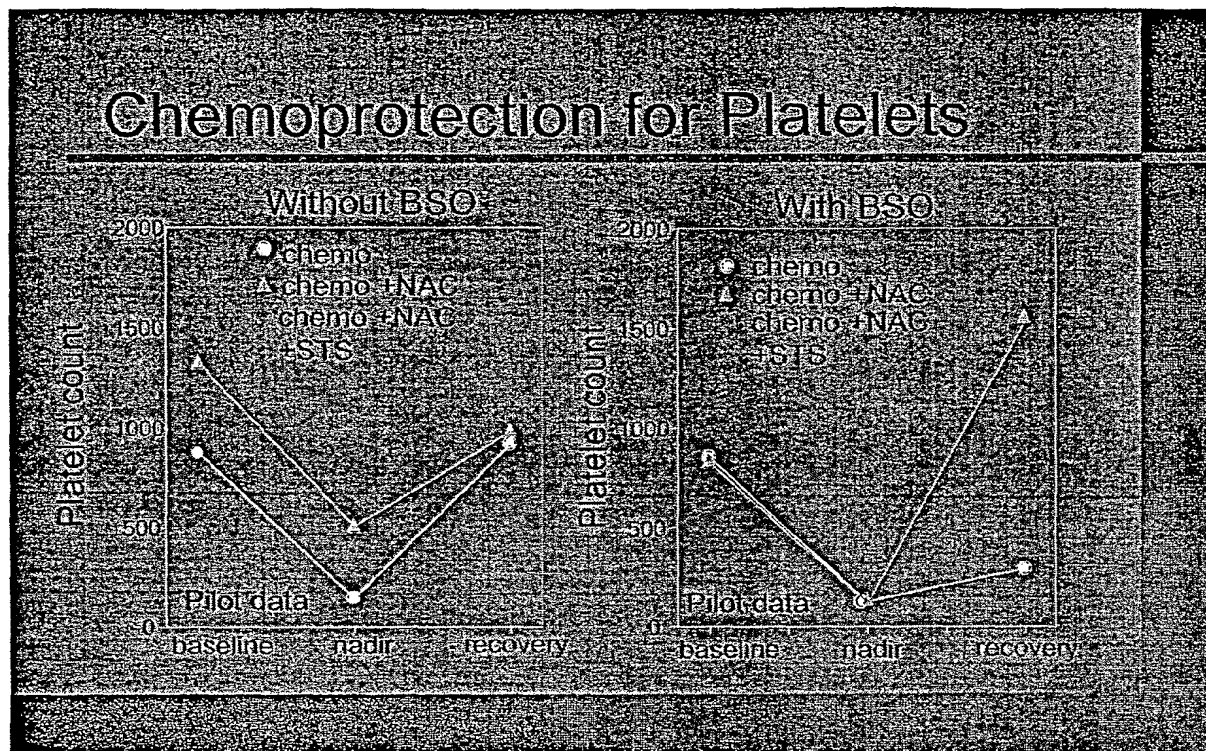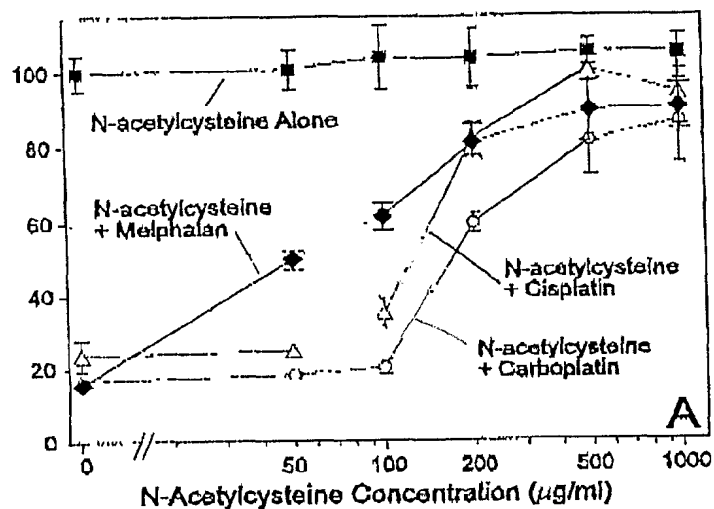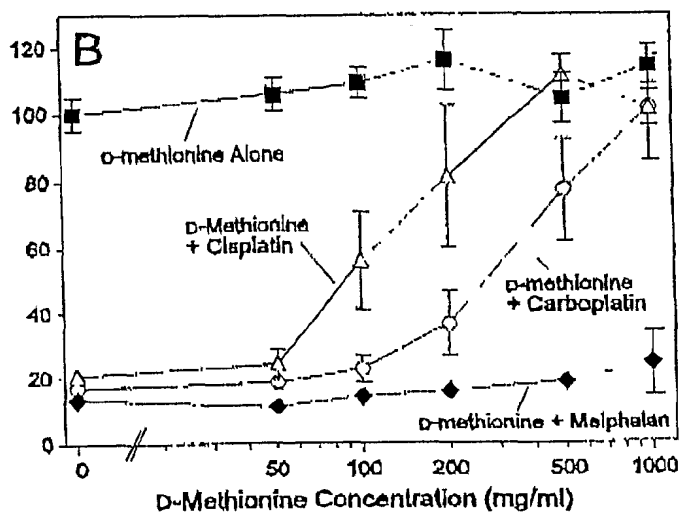
Arrows indicate direction
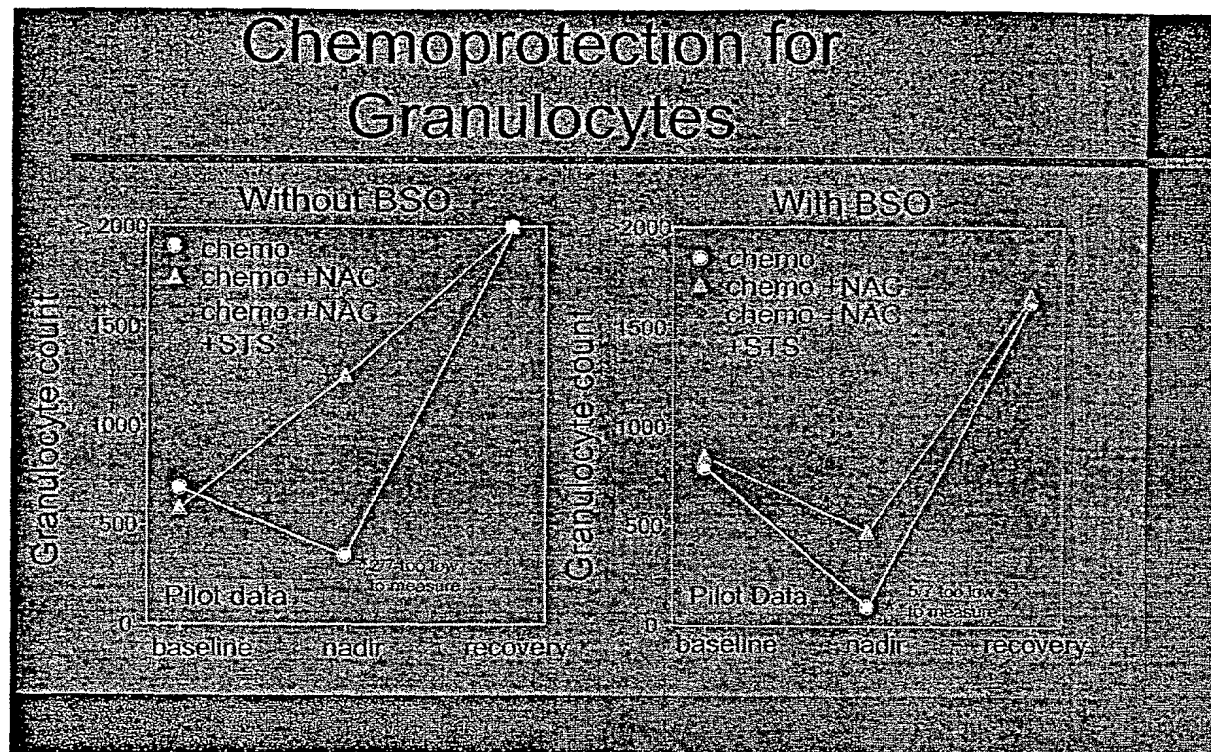of blood flow

level of catheter placement where
blow flow is distal to head and upper
extremities.

descending aorta

level of diaphram

hepatic arteries

renal arteries

Fig. 1

femoral
arteries

Fig. 2A

Fig. 2B

Fig. 2C

Fig. 3A

Fig. 3B
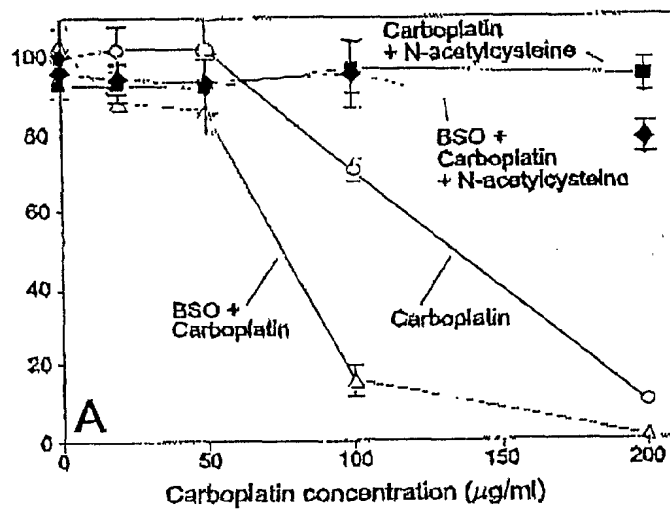
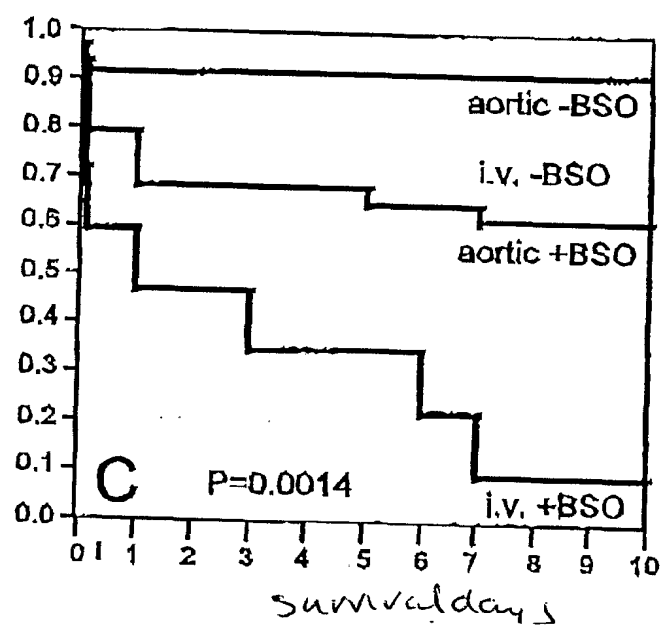**Fig. 4A**
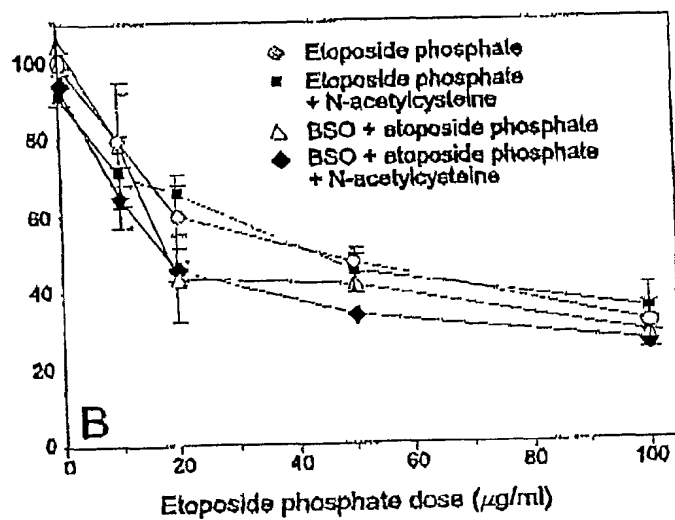
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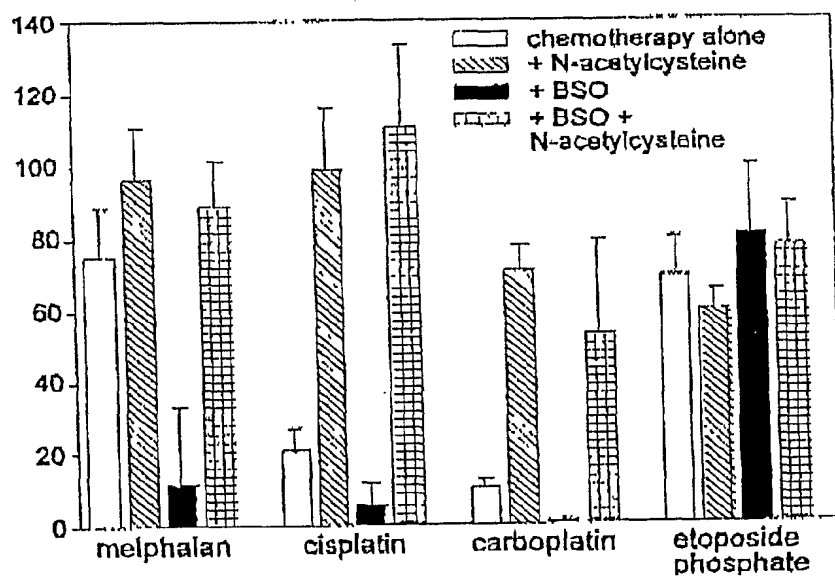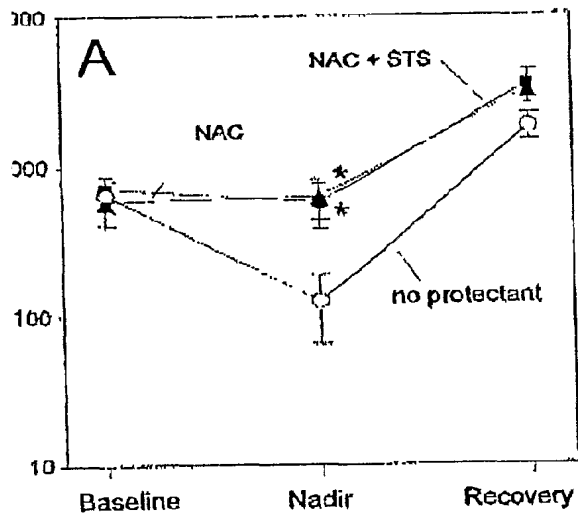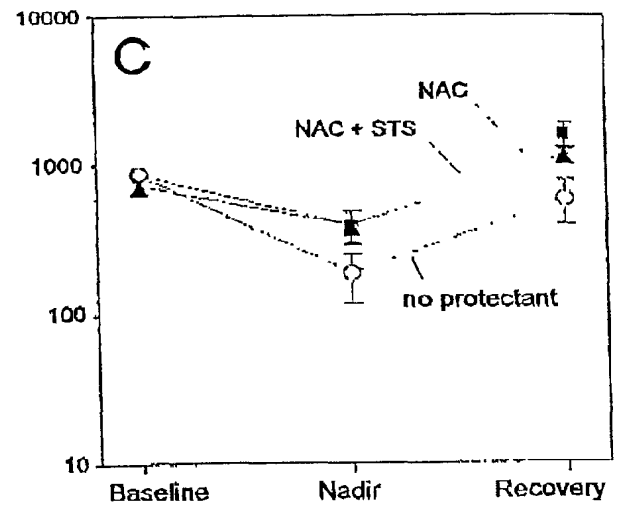
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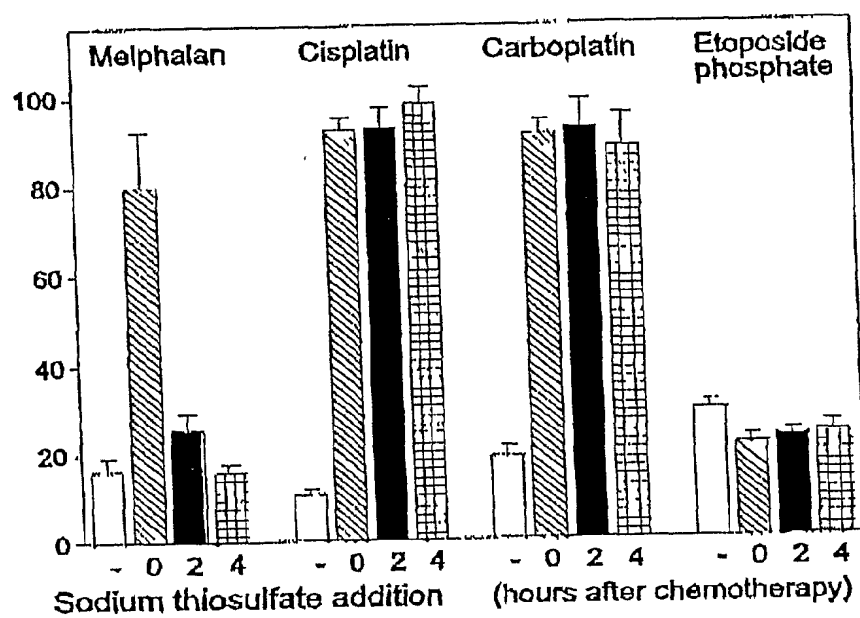
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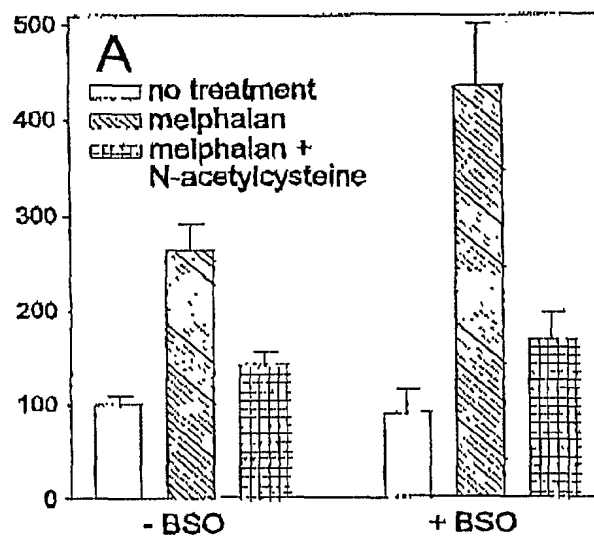
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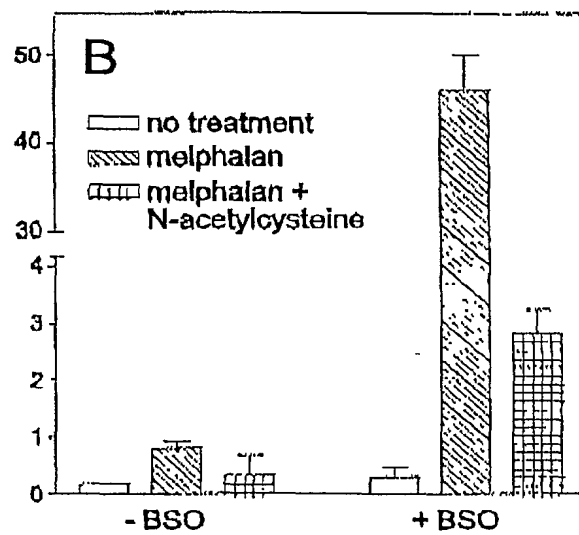
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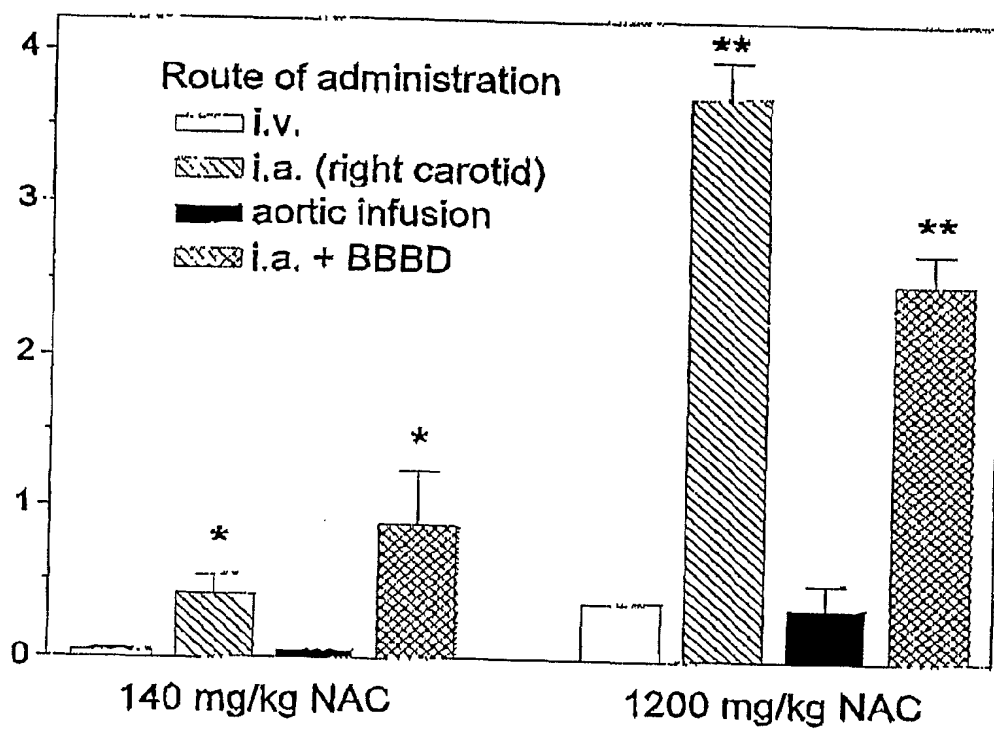
Fig. 8

Fig. 9

Fig. 10A

Fig. 10C

Fig. 10B

Fig. 10D